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Determination of ochratoxin A levels in ivorian cocoa beans intended for exportation

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ABSTRACT

The objective of this study is to monitor levels of ochratoxin A (OTA) in terms of the marketability of Ivorian cocoa beans stored at the ports of Abidjan and San Pedro. Thus, 270 samples of cocoa beans were analyzed. Merchantability and OTA levels were determined respectively according to the Ivorian Coffee and Cocoa stock exchange standards and the European Community regulation related to the analytical methods for the control of mycotoxins levels in foodstuffs. As regards merchantability, a significant difference at 5% risk was revealed between the values of moisture, graining and grades. Arithmetic means for humidity were respectively 6.7% and 6.5% for samples from the ports of Abidjan and San Pedro. The number of beans per 100 g of cocoa beans was 94 and 97 respectively for samples from ports of Abidjan and San Pedro. The classification in grades in terms of marketability indicates that respectively 87% and 89% of batches collected at ports of Abidjan and San Pedro can be marketed. Pertaining to OTA, no significant difference at 5% risk has been revealed between the concentrations found in samples from both ports and between different grades. OTA concentrations obtained averaged out at respectively 0.87 µg/kg and 0.94 µg/kg of cocoa beans for Abidjan and San Pedro. Merchantability criteria and OTA levels in cocoa beans were loosely linked in view of flimsy values of squared correlation coefficients.

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INTRODUCTION

Stakeholders in the cocoa sector agree that it is of particular interest that cocoa farmers apply good agricultural practices to warrant cocoa beans of prime quality. Cocoa beans are prone to quite a few post-harvest treatments that can be summarized in a pod breaking, fermentation, drying, packaging and storage before marketing. When these

treatments are poorly conducted, that leads inevitably to the appearance of flaws in the beans and, most worrying, the dissemination of toxin-producing molds such as *Aspergillus* responsible for the production of ochratoxin A (Bastide et al., 2006; Sackou et al., 2007). These defects (moldy, slaty, moth-eaten, germinated, flat, and high moisture levels) usually spring from attacks by insects or

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improper weather conditions (Bastide et al., 2006; Cocoqual, 2007; Pauline et al., 2008).

To ensure the marketability of cocoa for export, the Ivorian Coffee and Cocoa stock exchange has enacted rigid standards. Every consignment of cocoa which does not meet these rigorous standards is prohibited from leaving the country (BCC, 2009).

Several previous studies have pointed out the presence of ochratoxin A in cocoa and diverse byproducts (Miraglia and Brera, 2002; Burdaspal and Legarda, 2003; Bonvehi, 2004; Tafuri et al., 2004; Matsuoka, 2006). In Côte d'Ivoire, this mycotoxin (OTA) has been found in cocoa from both production areas and Ivorian ports (Laine, 2001; Dembele et al., 2009). We therefore undertook this study to assess the levels of ochratoxin A based on criteria of the marketability of cocoa beans for exportation.

MATERIALS AND METHODS

Sampling

The plant material used in this study consisted of cocoa beans collected from November 2007 to March 2008 at the ports of Abidjan (120 samples) and San Pedro (150 samples) according to the European Community regulation (EC, 2006).

Determination of cocoa beans marketability

According to Ivorian regulation (BCC, 2009), three parameters are taken into account when it comes to assess the merchantability of cocoa beans: the moisture content, the graining and the classification in grades (Table 1). The moisture content of cocoa beans was determined with the help of an oven at 105 °C until constant weight (AOAC, 1990). The graining was determined by counting the number of beans in 100 g of whole cocoa beans free of any foreign materials. Finally, the classification in grades was related to the percentage of defective cocoa beans for 100 g of whole cocoa beans. To this end, 100 g of whole cocoa beans were cut lengthwise through the middle with a bean knife brand STANLEY, to expose a maximum

cut surface of cotyledons. Visual examination of the inner of the two half-beans highlights possible flaws (ISO, 1977; Hamid and Lopez, 2000).

Determination of OTA levels in cocoa beans

Extraction of OTA

The entire sample was crushed in a hammer mill to obtain a homogeneous fine grind. In a Nalgene jar containing 15 g of homogenate, 150 mL of aqueous methanol-bicarbonate 1% (v/v, 50:50) were added. The mixture was homogenized by Ultra-Turax for 3 minutes and the homogenate was centrifuged at 5000 rpm for 5 minutes at 4 °C. The supernatant was filtered through filter paper into tubes of 25 mL. To 11 mL of filtrate were added 11 ml of saline phosphate buffered (PBS) at pH 7.3. Immunoaffinity columns brand Ochraprep and R-Biopharm were conditioned with 10 mL of PBS. Purification of 20 mL of the mixture was made on immunoaffinity columns and OTA extraction was performed with two volumes of 1.5 mL of PBS at a flow rate of 5 mL/minute. The resulting sample was packed in a chromatographic tube and the analysis of OTA was made by HPLC using the European community regulation (EC, 2006).

Apparatus

A liquid chromatograph HPLC brand Shimadzu coupled to a fluorescence detector was used and the operating conditions are described in Table 2.

Statistical analysis

The averages were calculated with their standard deviations to assess OTA levels, moisture content, graining and grades of cocoa beans. The squared coefficient of Bravais-Pearson was calculated to assess the correlation between OTA and the criteria for marketability. The homogeneity of means (OTA, moisture content and graining) was determined by a one or two-way analysis of variance (sampling location or period and/or grade) through Fisher test, using SPSS 12 software at 5% risk.

Table 1: Cocoa beans merchantability standard in Côte d'Ivoire.

Moisture content			
Maximum value allowed			8%
Number of beans per 100 g of cocoa beans			
Maximum value			105
Grades			
Grade	Moldy	Slaty	Defective Moth + Germinated + Flat
Grade I (GI)	≤ 3%	≤ 3%	≤ 3%
Grade II (GII)	> 3% and ≤ 4%	3% and ≤ 8%	> 3% and ≤ 6%
Low grade (LG)	> 4%	> 8%	> 6%

Table 2: HPLC analytical conditions.

Precolumn	Shim-pack GVP-ODS 10 x 4.6 mm
Column	Shim-pack GVP-ODS 250 mm x 4.6 mm
Detector	Fluorescence, λ excitation: 330 nm, λ emission: 460 nm
Mobile phase	Acetonitrile/Water/Acetic acid (99/99/2)
Injected volume	100 μ L
Flow rate	1 mL/minute
Column temperature	40°C
Rinsing solvent	Acetonitrile
Analysis duration	12 minutes

RESULTS

Merchantability of cocoa beans

The results of the marketability of cocoa beans are shown in Table 3. The average moisture contents were respectively 6.7% and 6.5% for the ports of Abidjan and San Pedro and 6.6% for all samples combined. The ports of Abidjan and San Pedro present respectively 7% and 1% of samples analyzed with a moisture content above the limit value of 8% set by Ivorian regulation (BCC, 2009). Results for graining indicated 94 and 97 beans per 100 g of cocoa beans for samples from the ports of Abidjan and San Pedro, which corresponds to 96 beans for both ports combined. The homogeneity of means between moisture contents and graining values performed by a one-way analysis of variance (sampling site), through Fischer-test revealed a significant difference at 5% risk. The classification in grades revealed that 87% and 89% of samples coming respectively from

the ports of Abidjan and San Pedro can be exported (Table 3).

Ochratoxin A levels in cocoa beans

Table 4 presents average values and ranges for OTA levels found in all samples from the ports of Abidjan and San Pedro. OTA average concentrations were respectively of 0.87 μ g/kg and 0.94 μ g/kg for samples collected in Abidjan and San Pedro that is 0.91 μ g/kg for both ports combined. OTA concentrations ranged between 0.05 and 4.79 μ g/kg for samples from Abidjan and 0.05 and 9.10 μ g/kg for those from San Pedro. The homogeneity of OTA concentrations which was performed by a one-way analysis of variance (sampling site), through Fischer test did not reveal a significant difference ($p < 0.05$) as shown in Table 4. Combined data from both ports showed 13% of samples with OTA concentrations exceeding the maximum limit value of 2 μ g/kg set by the European Union.

OTA levels in terms of cocoa beans grades

OTA levels in terms of cocoa beans grades are presented in Table 5. Grade I cocoa beans have average OTA levels respectively of 0.94 µg/kg and 0.89 µg/kg in the ports of Abidjan and San Pedro. Those classified Grade II have average OTA levels respectively of 0.80 µg/kg and 1.02 µg/kg in the ports of Abidjan and San Pedro. Low-grade cocoa beans presented on average 0.80 µg/kg and 0.95 µg/kg respectively in Abidjan and San Pedro. The homogeneity of the concentrations of OTA implemented by a two-way analysis of variance (Sampling site and grade) through Fischer-test did not reveal a significant difference p<5% as shown in Table 5.

Assessment of the variation of marketability criteria and OTA levels

We have monitored the variation of marketability criteria as well as OTA levels during a time period of 6 months (Table 6). OTA average concentrations ranged from 0.54 µg/kg to 1.11 µg/kg, moisture content ranged between 6.5% and 6.8%, and the number of

beans for 100 g of cocoa beans between 95 and 97 beans per 100 g. As for the grades, Grade I ranged from 3% to 51%, Grade II from 8% to 31% and the Low grade from 0% to 55%.

The homogeneity of OTA levels, moisture content and graining performed by a one-way analysis of variance (sampling period), through F-test, revealed for some criteria a significant difference at p < 5% (Table 6).

Correlation between OTA levels and cocoa beans marketability criteria

The correlation between merchantability criteria is very low except for the correlation between sprouted beans and flat ones (R2 = 0.145) and to a certain extent between moldy beans and sprouted ones (R2 = 0.052). The correlation between merchantability criteria and OTA levels is also weak. The tightest correlation was between OTA levels and the number of beans for 100 g of cocoa beans (Table 7).

Table 3: Marketability results.

Moisture content (%)				
Sampling sites	Average	Range	Percentage of sample* (%)	
			Moisture ≥ 8%	
Abidjan	6.7±0.7 a	5.6 - 9.9	7.0	
San Pedro	6.5±0.5 b	5.4 - 9.0	1.0	
Both ports	6.6±0.6 ab	5.4 - 9.9	4.0	
Graining (Number of beans for 100 g of cocoa beans)				
Sampling sites	Average	Range	Percentage of sample* (%)	
			Graining ≥ 8%	
Abidjan	94±5 a	75 - 105	0.0	
San Pedro	97±5 b	81 - 115	5.0	
Both ports	96±6 c	75 - 115	3.0	
Classification in grades (%)				
Sampling sites	Grade I (GI)	Grade II (GII)	Low-grade (LG)	Exportables (GI+GII)
Abidjan	48	39	13	87
San Pedro	57	32	11	89
Both ports	53	36	11	89

Means in a column, for each criterion, followed by the same letter are not significantly different (p < 0.05)

*Percentage of samples with moisture or graining levels exceeds the values shown.

Table 4: OTA concentrations in Ivory Coast cocoa beans.

Ports	Ota concentration (µg/kg)		Percentage of sample with OTA level > 2 µg/kg
	Average	Range	
Abidjan	0.87±0.81 a	0.05 - 4.79	11%
San pedro	0.94±0.92 a	0.05 - 9.10	15%
Both ports	0.91±0.90 a	0.05 - 9.10	13%

The averages in a given column followed by the same letter are not significantly different (p < 0.05)

Table 5: OTA levels in terms of Ivory Coast cocoa beans grades. Concentration: µg/kg).

Ports	Grade I (GI)	Grade II (GII)	Low-Grade (LG)
Abidjan	0.94±0.83 αα	0.80±0.95 αα	0.80±0.77 αα
San pedro	0.89±0.87 αα	1.02±1.01 αα	0.95±0.92 αα
Both ports	0.91±0.89 αα	0.91±0.87 αα	0.88±0.79 αα

Means followed by the same letters do not differ significantly (p < 0.05).

The letter a refers to a comparison of sampling sites while the letter α relates to a comparison of grades.

Table 6: Variation of marketability criteria and OTA levels.

sampling period	OTA level	Moisture	Graining	Grade I	Grade II	Low-Grade
	(µg/kg)	(%)	(Number of beans)	(%)	(%)	(%)
November	0.93±0.90 ab	6.8±0.6 a	95±8 a	26	23	55
December	1.11±1.01 a	6.5±0.6 b	97±5 a	51	26	7
January	0.73±0.69 b	6.6±0.6 ab	96±4 a	15	31	32
February	0.61±0.34 b	6.5±0.4 b	96±4 a	5	12	0
March	0.54±0.35 b	0.5±0.3 b	95±3 a	3	8	6
Total of each grade				100	100	100

In a given column, the averages followed by the same letter are not significantly different (p < 0.05).

Table 7: Correlation (R2) between OTA levels and cocoa beans marketability criteria.

	OTA levels	Graining	Moisture	GRADES				
				Moldy	Slaty	Moth-eaten	Flat	Sprouted
OTA levels	1.000	0.019	0.005	0.003	0.003	0.005	0.000	0.003
Graining		1.000	0.008	0.001	0.004	0.027	0.005	0.025
Moisture			1.000	0.002	0.000	0.000	0.006	0.007
Moldy				1.000	0.002	0.000	0.008	0.052
Slaty					1.000	0.013	0.036	0.022
Moth-eaten						1.000	0.000	0.023
Flat							1.000	0.145
Sprouted								1.000

DISCUSSION

Because of the greatest number of samples analyzed that met the requirements for export, the port of San Pedro turned out to be the port with cocoa beans of high marketability criteria levels. The rendering springing from the combination of marketability results from both ports is ambiguous: Ivorian cocoa beans marketability seems decreasing in comparison with the results of a previous study by Laine (2001), whereas it seems to have been improved compared to the results of Dembele et al. (2009). Indeed, Laine obtained an average moisture content of 7.6%, an average of 88 beans for graining and 93% of exportable beans, while Dembele et al. (2009) pointed out 63% of exportable beans for both Ivorian ports. For both ports the same trend is observed in the variation of marketability criteria and OTA levels. OTA levels in this study are relatively lower than the values obtained by Dembele et al. (2009), which were respectively of 0.70 µg/kg and 1.25 µg/kg for the ports of San Pedro and Abidjan.

The assessment of the variation of marketability criteria and OTA levels revealed that samples collected in December and January had the highest marketability. Moreover, OTA levels were most significant in cocoa beans collected in November and December. Hence, cocoa beans of prime quality are produced from December to January. Data from Dembele et al. (2009) study corroborate this fact. Nevertheless, no strong link was established between a poor marketability and OTA level, namely, a sample of Low Grade is not necessarily a sample with a high OTA level.

Conclusion

Altogether, this study showed an improvement in the marketability of cocoa beans intended for export. It also pointed out similar OTA levels in cocoa beans from both Ivorian ports (San Pedro and Abidjan). No direct relationship was established between the criteria of merchantability and OTA levels in cocoa beans. Thus, complying with

standard marketability criteria does not warrant a low OTA level in cocoa beans. Therefore, this study is interesting in the way that it makes available data on Ivorian cocoa beans marketability and on OTA levels as well. However, it will prove of paramount importance to conduct a similar study in production areas, and of particular interest to devise other criteria incorporating both marketability and sanitary quality of cocoa beans.

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